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Washington, DC 20059

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13. ABSTRACT ***(Maximum 200 Words)***

We have demonstrated that cell lines developed from metrizamide density fractions of peripheral blood hypodense (.22) and hyperdense (.24) eosinophils significantly inhibited UNCAP, PC3, DUl45 and HPCl monolayer growth cultures in vitro. This activity was enhanced when the eosinophils were pretreated with interleukin-5. DUl45 and PC3 colony formation was inhibited 50- 75% by eosinophil cell lines, while peripheral blood eosinophils inhibited PC3 colony formation by as much as 95%. 24hr. cultured eosinophil supernatants significantly inhibited PC3 colony formation with 3/7 supernatants causing 100% inhibition. Inhibitory activity of the 24hr .supernatants towards DUl45 was more variable, ranging from 9-90% inhibition of colony formation. There was slight enhancement of growth with one preparation. IL-4 and TNFα, which were found present in the supernatants also inhibited growth of monolayers cultures of PC3, DUl45 and HPCl. Flow cytometric analysis of adhesion molecule expression post; eosinophil:tumor cell co-culture, eosinophils 24hr culture supernatant treatment and exogenous cytokine treatment , revealed that 1) I-CAM was upregulated by IL-1, TNFα and IL-10, on DU145 and Pc3 and induced on UNCAP. ELAM-1 which was induced on PC3 by TNFα, IFNγ, IL-10 and IL-12, but not IL-4, VCAM-1 was induced by IL-10 only. Most significantly: the suppressor molecule E-cadherin was induced on both DU145 and PC3 by IL-12 which has been found in eosinophil 24hr supernatants. These data strongly support the hypothesis that eosinophils can destroy prostate tumor cells in vitro. The eosinophil cell lines and cytokine modulation of their activity offer an exquisite tool for more detailed study (both cellular and molecular) of a role for eosinophils as anti-prostate cancer effector cells.

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4. Introduction

Prostate cancer is the most common cancer diagnosed in American men. It has been estimated that by the end of 1999, 179,000 men will have been diagnosed with prostate cancer and that 37,000 deaths will have resulted(I). Prostate cancer incidence and mortality rates for African American males are the highest of any racial or ethnic group in the world (2). Prostate cancer incidence in this group is 180.6/100,000, compared to 143.7/100,000 for Caucasians and 24.2/100,000 for Koreans (2). The mortality rate for African American is 53.7 compared to 24.1 for Caucasians and 6.6 for Chinese.

Several new treatment approaches towards the eradication of prostate cancer have focused on regulating the immune response system to antigens expressed on prostate cancer cells (3- 7). Moreover the strategy of utilizing cytokine gene therapy in order to amplify the host response to tumor is quickly gaining momentum. Many of the cytokines which have been used (e.g. IL-2, IL-4, IL-5 and GM-CSF) are known to either attract and/or regulate eosinophil activity(8).

Eosinophils have been traditionally known as anti-helminthic effector cells and inflammatory agents in hypersensitivity reactions, particularly allergic asthma(9). Evidence exists, however, for a potential role for eosinophils in cancer. We have recently shown that activated eosinophils destroy MCF- 7 and MDA-231 breast cancer cell monolayer formation in vitro and inhibit MCF- 7 colony formation (manuscript in preparation). The inhibition observed is partially mediated by cytokines IL- 4 and *TNFa* which were secreted into 24-hr eosinophil conditioned supernatants. In this study, we have examined the inhibitory activity of activated eosinophils and eosinophilic cell lines which we had previously established and are presently characterizing (manuscript in preparation) on prostate cancer cell lines in eosinophil:tumor co-culture assays, and also the effect of cultured eosinophil supernatants on cell growth. Moreover this study investigates the potential regulation of cell adhesion molecule expression on prostate tumor cells. These molecules are involved in the migration of cells.

5. Body

Propagation of Cell Lines: To date all six eosinophilic cell lines have been retrieved ftom storage at -160°C, cultured in RPMI medium supplemented with penicillin/streptomycin (50 units/50 ug/ml respectively), gentamycin (50ug/ml) and 10% fetal bovine serum. We have data with 3 of the cell lines and 2 sublines. Tumor: PC-3, DU145 and LNCaP cells were obtained from ATCC and established in culture, fozen and retrieved prior to use. They were being maintained in the appropriate culture medium as recommended by the vendor; PC3 (7% F-12K medium supplemented with penicillin/streptomycin and gentamycin); DU145 and LNCAP in 10% RPMI medium supplemented with penicillin/streptomycin and gentamycin. In a collaborative study, we at Howard University have very recently immortalized a primary prostate culture HPCl ftom an African American. This cell line is presently being characterized. These cells are also cultured in 15% RPMI medium containing penicillin/streptomycin and gentamycin and were used preliminarily in this study.

Growth Inhibition of PC3, LNCaP, DUl45 and HPCl Tumor Cells by Activated Eosinophils and Eosinophilic Cell Lines.

A. Monolayer. Tumor Cells (pC3, LNCAP , DU145 and HPCl were seeded into 6-well plates (at $2.5x10^5$ cells per well) or 12-well plates (at $1.5x10^5$ cells per well). The plates were incubated overnight (16-24hr) at 37°C. Eosinophils were added at various effector to target (E:T) ratios and the plates incubated for an additiona124-48hr. Effector cells were then removed, the mono layers washed 3x with PBS and stained with H&E.

LNCAP was extremely sensitive to hypodense eosinophils at 5:1 and 43:1 E:T ratios and hyperdense eosinophils at 5:1 and 14:1 E:T ratios. PC3 was also sensitive to killing by eosinophil hypo- and hyperdense cell lines.

Eosinophil cell lines were sterile sorted with a Becton Dickinson F ACS SCAN Cell Sorter using the PE-labeled antibody to the eotaxin receptor. This chemokine receptor is found predominantly on eosinophils. These sublines were found also to be positive for the eosinophil markers CD15 and CD49d. Both the parent eosinophil cell line GRC.014.24 and the two sublines GRC014.24.S1 and GRC.014.24.S2 markedly inhibited PC3 cell growth.

When 24hr .cultured eosinophil supernatants were added to subconfluent PC3 and DU145 monolayers cell growth was dramatically inhibited.

B. Colony Formation. PC3 and DU145 cells were seeded into the wells of 6-well tissue culture plates at 100 cells per well. The plates were incubated overnight at 37°C,5% CO2. At this time effector cells were added at various E:T ratios and the plates were incubated for ten days. The plates were harvested, washed 3x with PBS, then stained with H&E and counted manually. Both hypo- and hyperdense subpopulations of peripheral blood eosinophils inhibited PC3 colony formation in a dose-dependent manner, with the 50:1 E:T ratio resulting in 95% inhibition for the hypodense eosinophils and 91% inhibition for the hyperdense eosinophils. The cell line GRC.014.24 inhibited colony formation by 71 and 75% at E:T ratios 1:2 and 2:1, respectively. At the E:T ratio of 2:1, GRC.014.24 inhibited DU145 by 88% and the sublines SI and S2 inhibited colony formation by 81 and 54%, respectively. The hypodense cell line BJA.060.22 inhibited colony formation by 50%.

C. 24hr. Cultured Supernatants Inhibit Prostate Tumor Cell Growth In Vitro. Subconfluent mono layers of PC3, DU145 and HPCI prostate cells were incubated overnight with 24-hr. eosinophil cultured supernatants in 12-well tissue culture plates. Both hypodense and hyperdense eosinophil cultured supernatants markedly inhibited PC3 colony formation and at least three supernatant preparations {BLA 24, HMO 22 and HMO 24)

completely prohibited colony formation. GRC.014.24 supernatant completely inhibited DU145 colony formation.

D .Cytokine Presence in 24hr .Eosinophil SUDernatants. 24hr .cultured supernatants from peripheral blood eosinophil hypodense and hyperdense subpopulations M22 and M24, respectively) were evaluated by enzyme-linked immunoassay (ELISA) analysis using commercial kits. Interleukin- 4 (IL-4) and Tumor Necrosis Factor Alpha (TNFα) were present in varying levels in all individuals tested (Table I). IL-4 concentrations ranged from 0 to >1000 pg/ml. TNFα concentrations were far less than IL-4, ranging from 10-224pg/ml.

E. Baseline Expression of Adhesion Molecules, {E-selectin, ELAM, ICAM-1, VCAM-1, VLA-4) on PC3, DU145 and LNCAP prostate cell lines. All cell lines were subcultured with their appropriate media (PC3 - F12K complete with 7% FBS; DU145 and LNCAP -RPMI complete with 10% FBS). Optimum incubation time and temperature was determined for those adhesion molecule antibodies that were not tested for flow cytometry use prior to purchase. Those antibodies purchased from Becton Dickinson, or comparable companies specializing in flow cytometry reagents, were used according to vendor specifications. The adhesion molecules tested were E-Selectin, ICAM-1, VCAM-1, and VLA-4. Moreover E-Cadherin and N-Cadherin expression were tested on PC3 and DU145. ELAM, ICAM-1 and VCAM-1 were examined by direct flow cytometric procedures and E-Cadherin, N-Cadherin and VLA-4 were analyzed by indirect flow cytometry, according to vendor's protocol.

6. Key Research Accomplishments

- Retrieval of all eosinophilic cell lines
- Demonstration of functional cytotoxic/cytostatic activity with 3 of the lines and 2 sublines
- Establishment of new prostate cell line in collaboration with clinical investigators at Howard University Hospital
- Use ofnew prostate cell line in eosinophil co-culture assays.
- Determined cytokine modulatory effects on cell adhesion molecules ~ Upregulated tumor suppressor E-Cadherin in PC3 cells

7. Reportable Outcomes

Promotion from Assistant Professor to Associate Professor.

Activated eosinophils inhibit In .Vitro growth of prostate cancer cell lines, {Manuscript to be submitted) May/June)

Late Abstract for AACR Spring 2000, (Eosinophil Cell Lines Inhibit Prostate Cancer Cell Growth In Vitro).

Ahaghotu C, Marshalleck J, Dennery M, Vaughn T, Laniyan I, Jackson A, **Furbert-Harris P.** A Novel Primary Prostate Cancer Cell Line Derived from an African American Patient. The American Urological Association, Inc. 95th Annual Meeting, 1999.

Poster presentations at The AACR Special Conference on Cytokines in Cancer, September 2000.

Paulette M. Furbert-Harris, Ibrahim Laniyan, Keith A. Hunter, Theresa R. Vaughn, Debra Parish-Gause, Kesha C Forrest, Lanette Brooks, Reisha Albury, Christina Howland, Josephine Okomo-awich, and Oladipo A Oredipe. Regulation of E-cadherin Expression on Prostate Cancer Cells by Activated Eosinophils is Mediated by IL-12. AACR Proceedings, Cytokines and Cancer: Regulation, Angiogenesis, and Clinical Applications, 2000.

8. Discussion/Conclusions

We hypothesized that activated eosinophils which may be found in tumor infiltrates produce cytokines which are both tumor inhibitory and enhancing. Moreover these cytokines may modify adhesion molecule expression on tumor cells thereby modifying their mortality and metastatic capabilities. The tasks for the 18 month period.

a. culturing and propagation of both prostate cells and eosinophilic cell lines. b. growth inhibition assays (monolayer/colony). c. cytokine enhancement of eosinophil activity .

d. exogenous cytokine activity against prostate tumor cell growth.

e. flow cytometric analysis of adhesion molecule expression post eosinophil:tumor cell co culture.

f. the modulation of adhesion molecules were studied by flow cytometry .

g. effect of eosinophil supernatants with and without coculture on adhesion molecule expression on prostate tumor cells.

h. exogenous cytokine modulation of adhesion molecules on prostate tumor cells.

The data presented have clearly shown that subpopulations of activated eosinophils, (hypodense and hyperdense) from individuals with mild to moderate eosinophilia inhibit the growth ofPC3, tumor cells (both monolayer and colony formation). Furthermore eosinophil cell lines established from these subpopulation inhibited LNCAP , PC3, DU145 and the newly established prostate cell line HPC 1. In the co lony assay PC3 was more sensitive than D U 145 , to eosinophil killing. IL-5 enhanced hypo dense and hyperdense cell line killing of both PC3 and HPC 1 tumor cells. Both LNCAP and HPC 1 failed to form colonies and hence we simply used monolayers to test eosinophil activity. IL-5 were the only cytokine used thus far to enhance eosinophil activity.

ICAM-l was expressed on DU145 and PC3. This was upregulated by IL-l, TNFα and IL-I0. ICAM-l was induced on LNCaP by ILIα and TNFα. ELAM-l and VCAM-l were absent on PC3 but ELAM -1 expression was induced by TNFα, IFNγ, IL-l 0 and IL-12, but not IL-4 and V CAM -1 was induced by IL-l 0 only. TNFα and IL-4, induced marginal ELAM-l expression on DU145, not LNCAP .The most significant observation has been the induction of the suppressor adhesion molecule E-Cadherin by IL-12, and eosinophil enhancement of E-Cadherin on both DU145 and PC3. To this investigator's knowledge this has not been reported in prostate cancer and the only other report has been by Hiscox et al with human colon cancer cells, (Clin Exp Metastasis 13(5): 396-404 (1995). IL-12 is well known for its immunomodulatory activities. IL-12 is now being vigorously studied as an anticancer cytokine. The observation presented in this report reaffirms the potential significance of IL-12 as an anticancer therapeutic agent. Eosinophils produce IL-12, hence this cell gains continuing attention as an anticancer effector and the use of eosinophil cell lines which we have developed offer exquisite research tools for clearly defining and for strategically manipulating with cytokine in order to maximize their worth as anticancer effector agents.

9. References

1. American Cancer Society (1998): Facts and figures.

2. Brawley OW and Kramer BS. Comprehensive textbook of Genitourinary Oncology. *NI* Vogesang, PT Scardino, WU Shiplay, OS Coffey Eds. (Williams and Wilkins, Baltimore, 1996.

3. Tjoa BA, Erickson *SI,* Bowes V A, Rag de H, Kenny GM, Cobb OE, Ireton RC, Troychak *MI,* Boynton AL and Murphy GP. Follow-up evaluation of prostate cancer patients infused with autologous dendritic cells pulsed with PSMA peptides. Prostate 32:272-278, 1997.

4. Tjoa BA, Simmons *SI,* Bowes V A, Radge H, Rogers M, Elgamal A, Kenny GM, Cobb OE, Ireton RC, troychak *MI,* Salgaller ML, Boynton AL and Murphy GP. Evaluation ofPhase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides. Prostate 36: 39- 44, 1998.

5. Zhang S, Zang HS, Reuter YE, Slovin SF, Scher ill and Livingston PO. Expression of potential target antigens for immunotherapy on primary and metastatic cancers. Clin Cancer Res 4: 295~302, 1998.

6. Correale P, Walmsley K, Zaremba S, Zhu M, Schlom *I* and Tsang KY. Generation of human cytolytic T lynlphocyte lines directed against prostate-specific antigen (PSA) employing a PSA oligopeptide. *I* ImmunoI161:3186-3194, 1998.

7. Simons JW and Mikhak B. Ex vivo gene therapy using cytokine-transduced tumor vaccines: molecular and clinical pharmacology. Semin Oncol25: 661-676, 1998.

8. Silberstein DS. Eosinophil function in health and disease. Critical Reviews in Oncology!Hematoloy 19(1995): 47-77,1994.

9. Gaga M, Frew AJ, Veronica AV and Kay AB. Eosinophil activation and T lymphocyte infiltration in allergen-induced late phase skin reactions and classical delayed-type hypersensitivity. IImmuno1147: 816-822,1991.

10. Appendices.

Figure 1. Inhibition ofLNCAP Tumor Cell Growth by Eosinophil Cell Lines Figure 1. Legend

Figure 2. Inhibition ofPC3 Tumor Cell Growth by Eosinophil Cell Lines Figure 2. Legend

Figure 3. IL-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition ofPC3 Tumor Cells In Vitro
Figure 3. Legend

Figure 4. IL-5 Treatment of Eosinophil Cell Lines Enhances Growth Inhibition ofPC3 Tumor Cell In Vitro Figure 4. Legend

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Figure 11. HPC 1 Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines Figure 11. Legend

Figure 12. HPCl Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines Figure 12. Legend

Fig. 1. Inhibition of LNCaP Tumor Cell Growth by Eosinophil Cell Lines

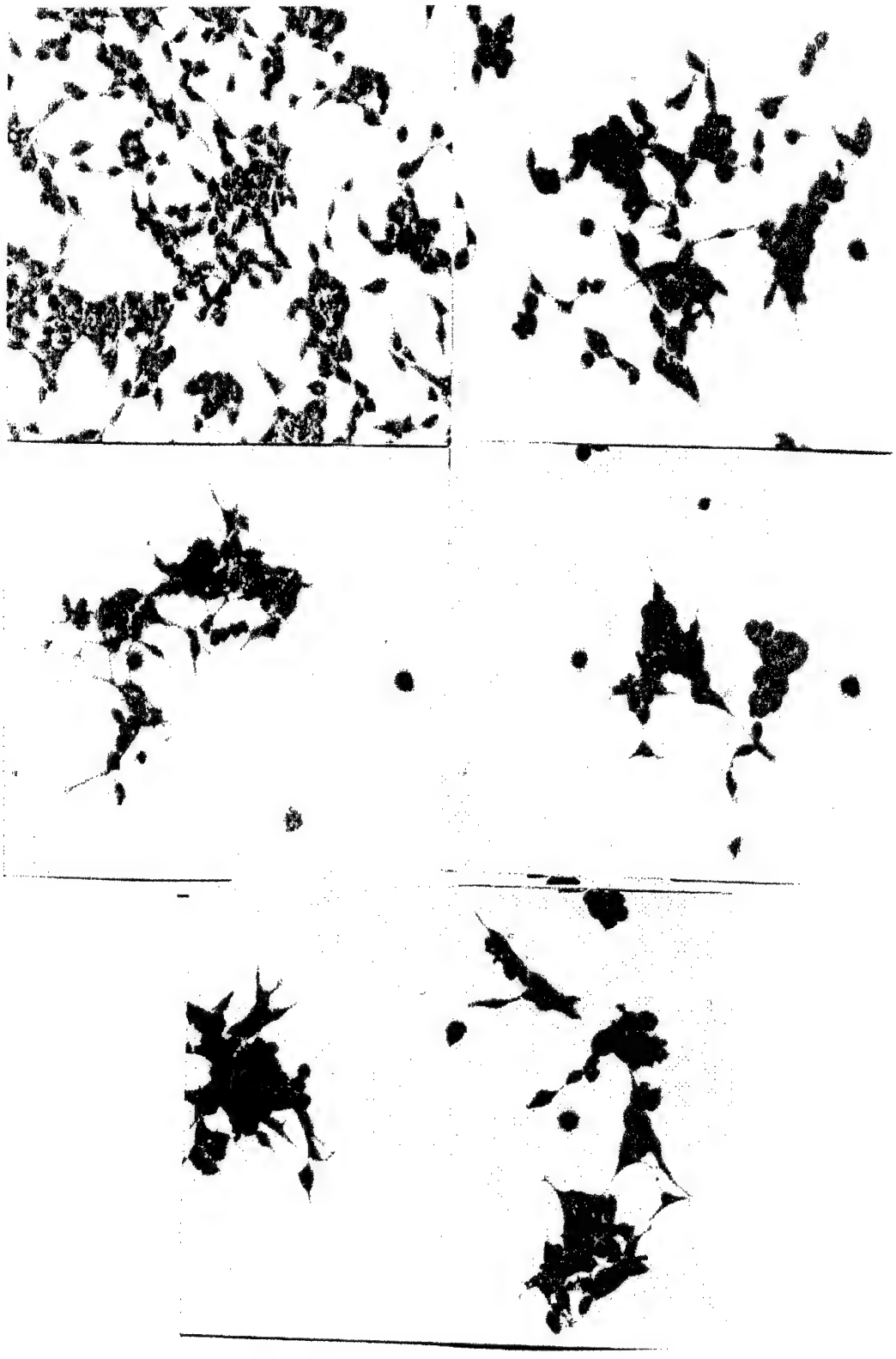


Fig. 1. LNCaP tumor cells were seeded into T25 flasks at 3×10^5 cells/flasks and allowed to grow to confluence (3-4 days) in media alone (A) or in the presence of hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B&C, respectively) and hyperdense cell line (SD.031.24) at E:T ratios of 5:1 and 14:1 (D and E, respectively). All experiments were performed in duplicate.

Fig. 2. Inhibition of PC3 Tumor Cell Growth by Eosinophil Cell Lines

Fig. 2 PC-3 tumor cells were seeded into duplicate T25 flasks at 3×10^5 cells/flask and allowed to grow to confluence (3-4 days) in media alone, and in co-culture with hypodense eosinophilic cell line SD.031.22 at E:T ratios of 5:1 and 43:1 (B, C, respectively) and hyperdense cell line SD.031.24 at E:T ratios of 5:1 and 14:1 (D & E, respectively).

Fig. 3. Interleukin-5 Pretreatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro

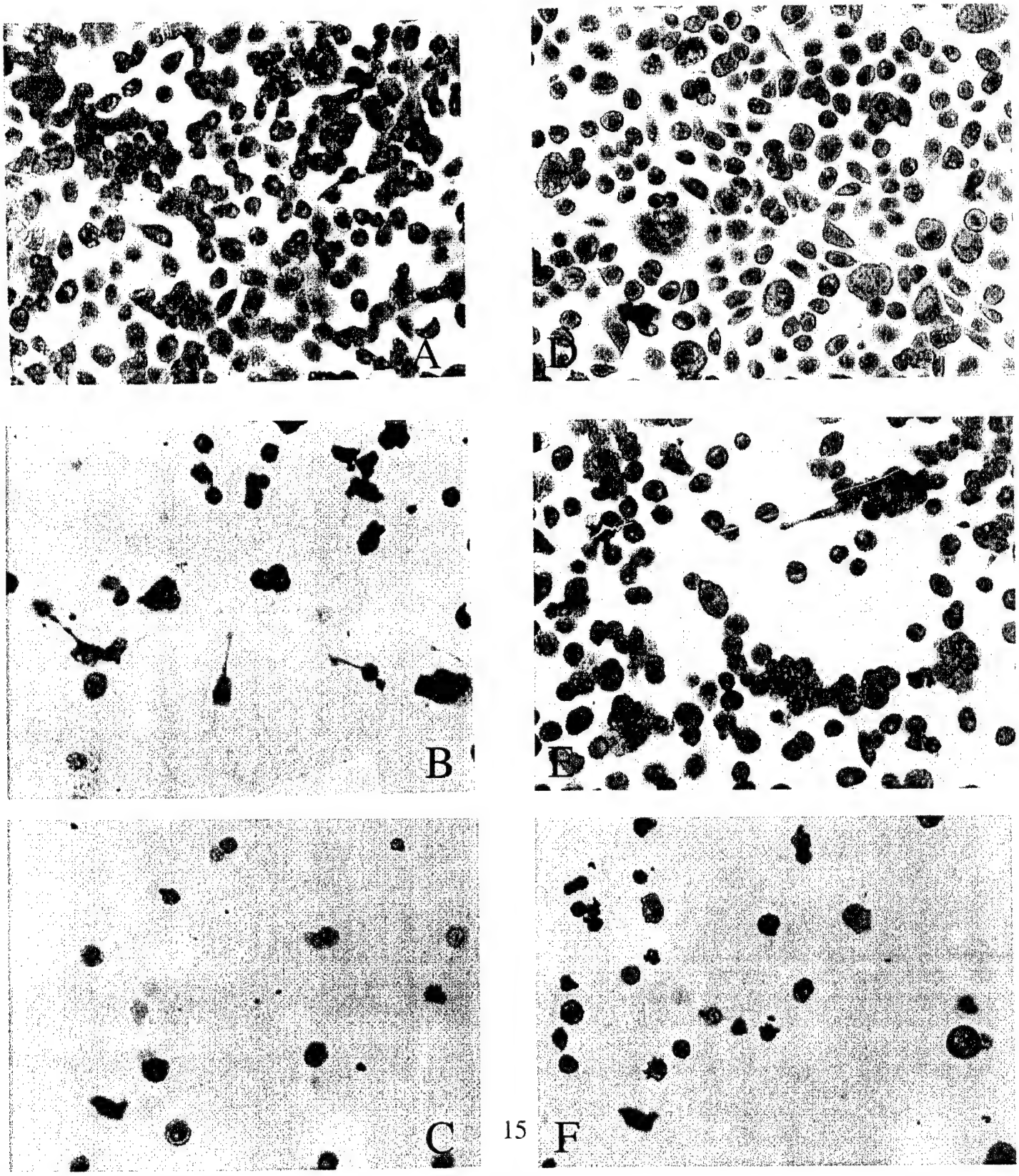
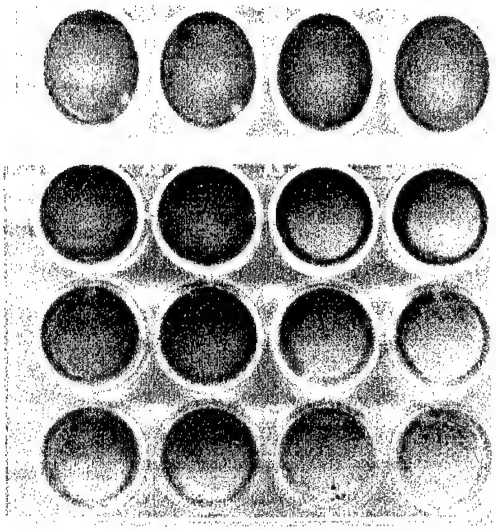


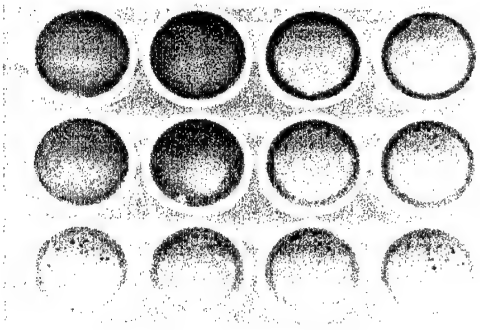
Fig. 3. PC-3 tumor cells were seeded into the wells of a 12 well culture plate at 1.5×10^5 cells/well. Prior to this effector eosinophil cell lines were pretreated with IL-5 (l ng/ml) for 3 days. On day 4, eosinophils were added and the plate incubated for 24hr or until the control wells were confluent (24-48hrs.). Effector cells were removed and the wells washed 3X with PBS, then fixed and stained with H&E. Photomicrographs were taken as well as a scan of the entire well or plate.

Fig. 4. IL-5 Treatment of Eosinophil Cell Lines Enhances Growth Inhibition of PC3 Tumor Cells In Vitro

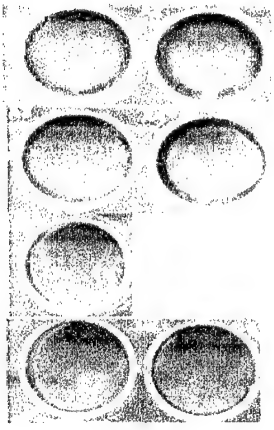


Control

A1-2: GRC014"24":PC-3, 1:1
B1-2:GRC014"24":PC-3, 10:1
C1-2:GRC014"24":PC-3, 25:1
A3-4: GRC014"24"+IL-5:PC-3, 1:1
B3-4: GRC014"24"+IL-5:PC-3, 10:1
C3-4: GRC014"24"+IL-5:PC-3, 25:1



A1-2: BJA060"22":PC-3, 1:1
B1-2:BJA060"22":PC-3, 10:1
C1-2:BJA060"22":PC-3, 25:1
A3-4: BJA060"22"+IL-5:PC-3, 1:1
B3-4: BJA060"22"+IL-5:PC-3, 10:1
C3-4: BJA060"22"+IL-5:PC-3, 25:1



WCH"22", 5:1(Peripheral Blood Eosinophils)

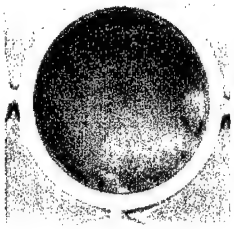
WCH"22", 10:1(Peripheral Blood Eosinophils)

WCH"22", 25:1 (Peripheral Blood Eosinophils)

WCH"24",5:1 (Peripheral Blood Eosinophils)

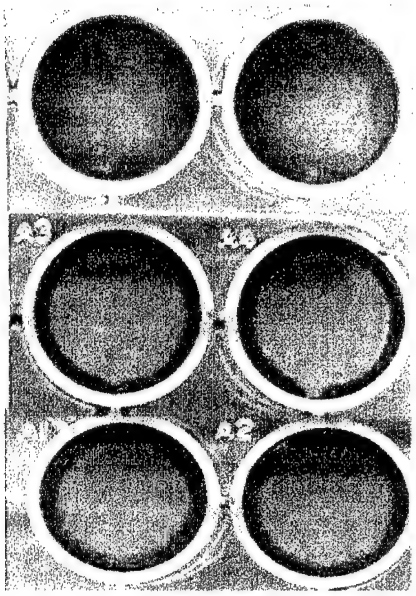
Fig. 4. Eosinophils, both peripheral blood and eosinophil cell lines were co-cultured in duplicate wells of a 12-well tissue culture plate with PC3 tumor cells at E:T ratios 1:1, 10:1 and 25:1 as described in fig. 3. The plates were harvested and stained with H & E then scanned into power point for presentation. The alpha numeric represents the donor and the numbers in quotations represent the eosinophil subpopulations (22-hypodense and 24-hyperdense).

Fig. 5. CD15 and CD49d Positive Eosinophil Sublines Inhibit PC3 Cell Growth In Vitro



Control

Panel A



Panel B

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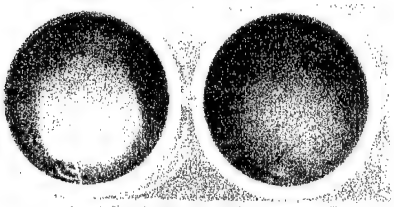

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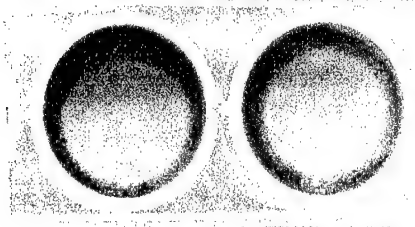

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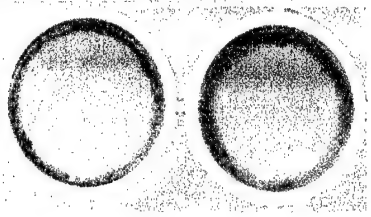


Panel C

3

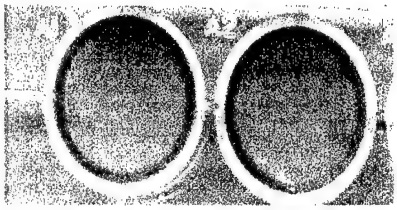
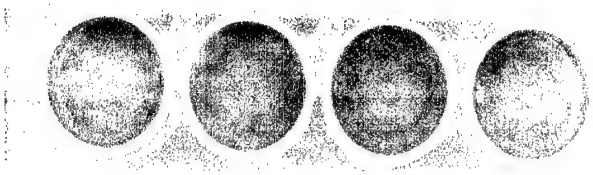
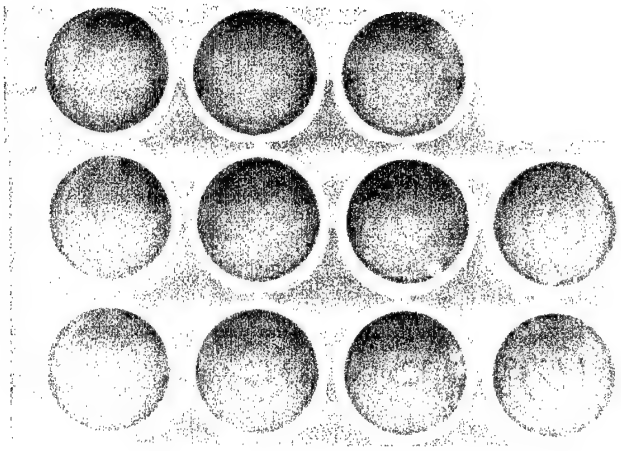


Fig. 5. Sublines from the eosinophil parent line GRC.014.24 were sterile-sorted with a FACS SCAN cell sorter using antibody to the eosinophil specific eotaxin receptor. The sublines S1 and S2 were found to be positive for both CD15 and CD49d markers. The co-culture was set up similarly to that described in figures 3 and 4. Numbers 1, 2 and 3 represent E:T ratios 1:2, 2:1 and 5:1, respectively.

Fig. 6 24-hr Cultured Eosinophil Supernatants Inhibits PC3 Cell Growth In Vitro

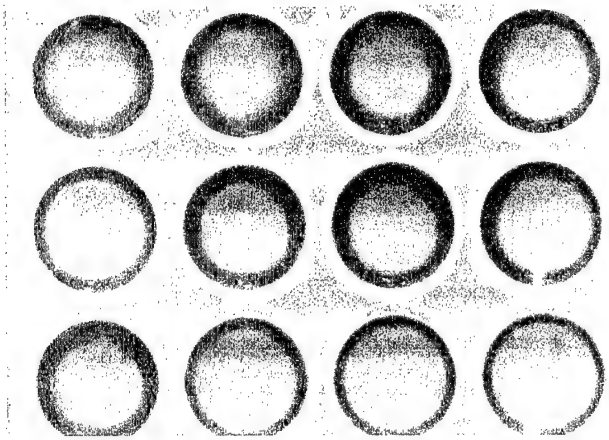


Control



A1-2: BLA"22"
A3: BLA"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"

GRC.014.24



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 6. PC-3 tumor cells were incubated (1.5×10^5 cells/well) overnight at 37°C. Duplicate wells were then treated with 24hr. cultured supernatants from peripheral blood eosinophil hypodense (22) and hyperdense (24) subpopulations, from donors BLA, HMO, YDA and WCH. Tumor 1cells were also treated with IL-4 and TNFα at 10, 50 and 100ng/ml 24-48hr later.

Fig. 7. Eosinophil Inhibit PC-3 Colony Formation In Vitro

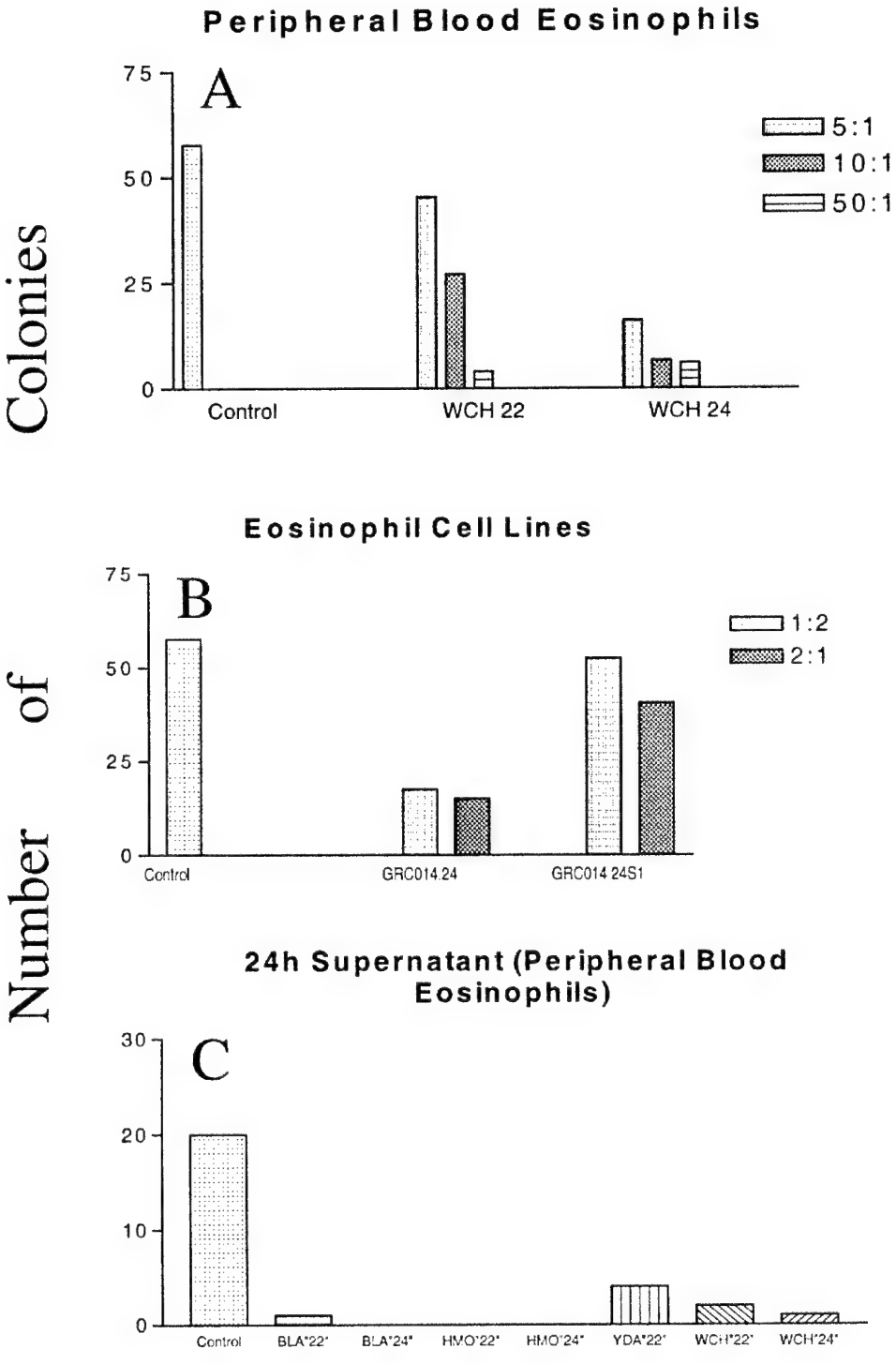
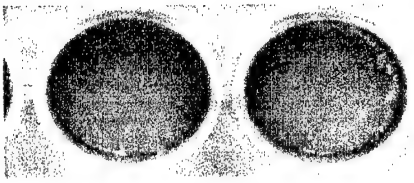
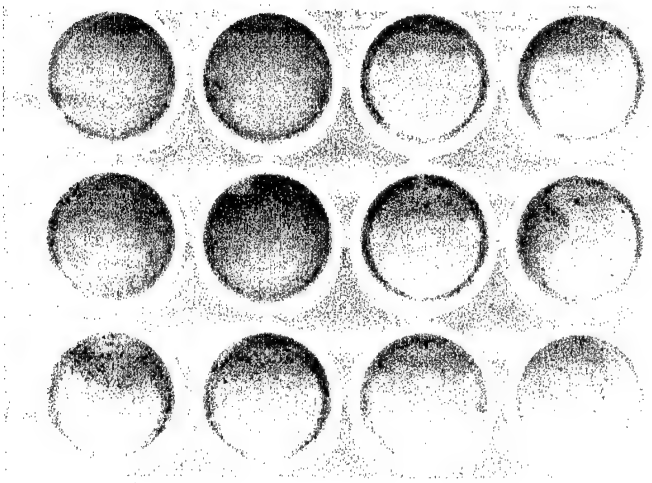


Fig. 7. PC-3 cells were seeded into duplicate and sometimes triplicate wells of a 6-well plate at 100 cells per well. After 24hr incubation eosinophils [fresh peripheral blood eosinophils (panel A); eosinophil cell lines (panel B)], and cultured supernatants (panel C) from both peripheral blood eosinophils (WCH 22 and WCH 24) and eosinophil cell line (GRC.014.24.2) and the plates were further incubated for 10 days. The plates were then harvested, stained with H & E and the colonies counted manually.

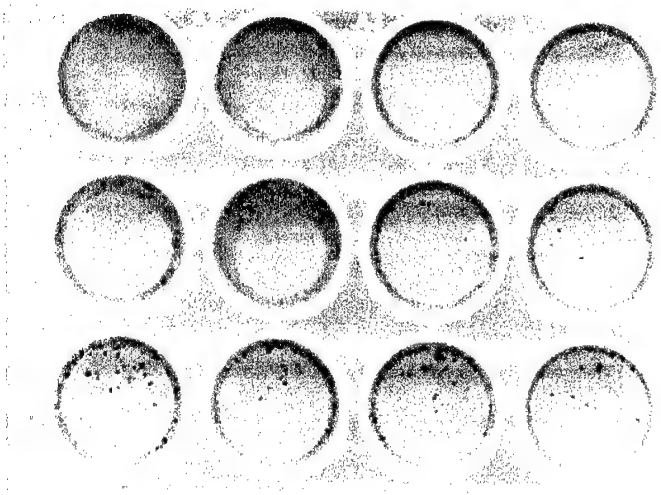
Fig. 8 Eosinophil Cell Lines Inhibit DU145 Cell Growth In Vitro



Control



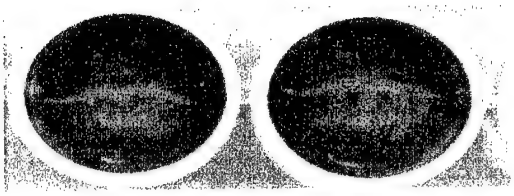
A1-2: BJA060"22":DU145, 1:1
B1-2: BJA060"22":DU145, 10:1
C1-2: BJA060"22":DU145, 25:1
A3-4: BJA060"22"+IL-5:DU145, 1:1
B3-4: BJA060"22"+IL-5:DU145, 10:1
C3-4: BJA060"22"+IL-5:DU145, 25:1



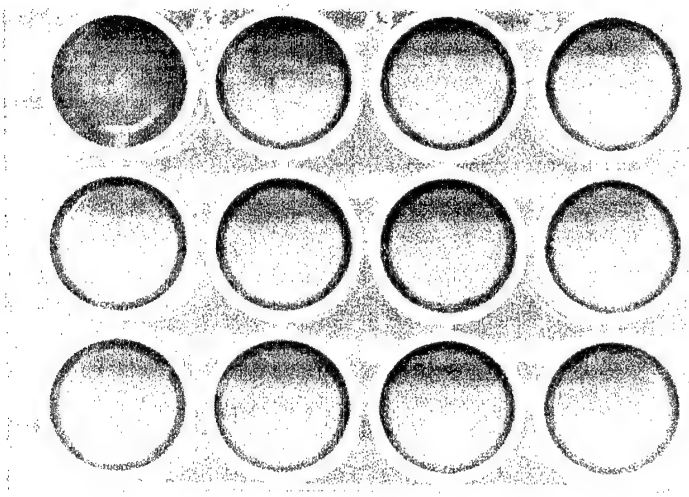
A1-2: GRC014"24":DU145, 1:1
B1-2: GRC014"24":DU145, 10:1
C1-2: GRC014"24":DU145, 25:1
A3-4: GRC014"24"+IL-5:DU145, 1:1
B3-4: GRC014"24"+IL-5:DU145, 10:1
C3-4: GRC014"24"+IL-5:DU145, 25:1

Fig. 8. DU145 prostate cells were seeded into duplicate wells of a 6-well plate at 1.5×10^5 cells/well and incubated overnight at 37°C . IL-5 treated and untreated eosinophil cell lines were added at E:T ratios 1:1, 10:1, and 25:1. The plates were incubated for an additional 24-48hr. Effector cells were removed, the plates were washed 3x with PBS then fixed and stained with hematoxylin and eosin. The entire plate or individual wells were then scanned into power point
for presentation.

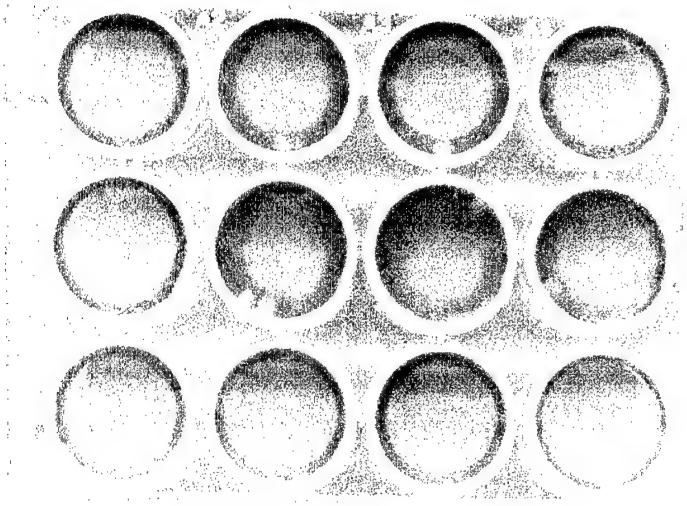
Fig. 9. 24hr Cultured Supernatants Inhibit DU145 Cell Growth In Vitro



Control



A1-2: BLA"22"
A3: BLA"24"
A4: HMO"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"



A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

Fig. 9. DU145 cells ($1.5x10^5$) were treated with 24hr. cultured supernatants from various donor peripheral blood eosinophil subpopulations. Cells were also treated with IL-4 and TNFα at 10, 50 and 100ng/ml. The plates were stained with H & E and scanned into power point for presentation.

Table 1. CYTOKINE CONCENTRATIONS IN 24HR EOSINOPHIL CULTURE SUPERNATANTS (pg/ml)

Donor	IL-4		IL-5		TNFα		GM-CSF	
	22	24	22	24	22	24	22	24
1	>1000	>1000	440	435	50	63	0	0
2	316	3	0	0	100	56	0	0
3	>1000	631	0	0	50	16	0	0
4	>1000	0	nt	nt	129	200	nt	nt
5	200	20	0	0	100	224	nt	nt
6	8	>1000	0	186	10	7.9	450	450

Table 1. 24hr conditioned supernatants were tested for cytokines IL-4, IL-5, TNFα and GM-CSF using commercial enzyme linked immunoassay kits.

Fig. 10. DU145 Colony Inhibition by Eosinophil Cell Lines and Cultured Supernatants

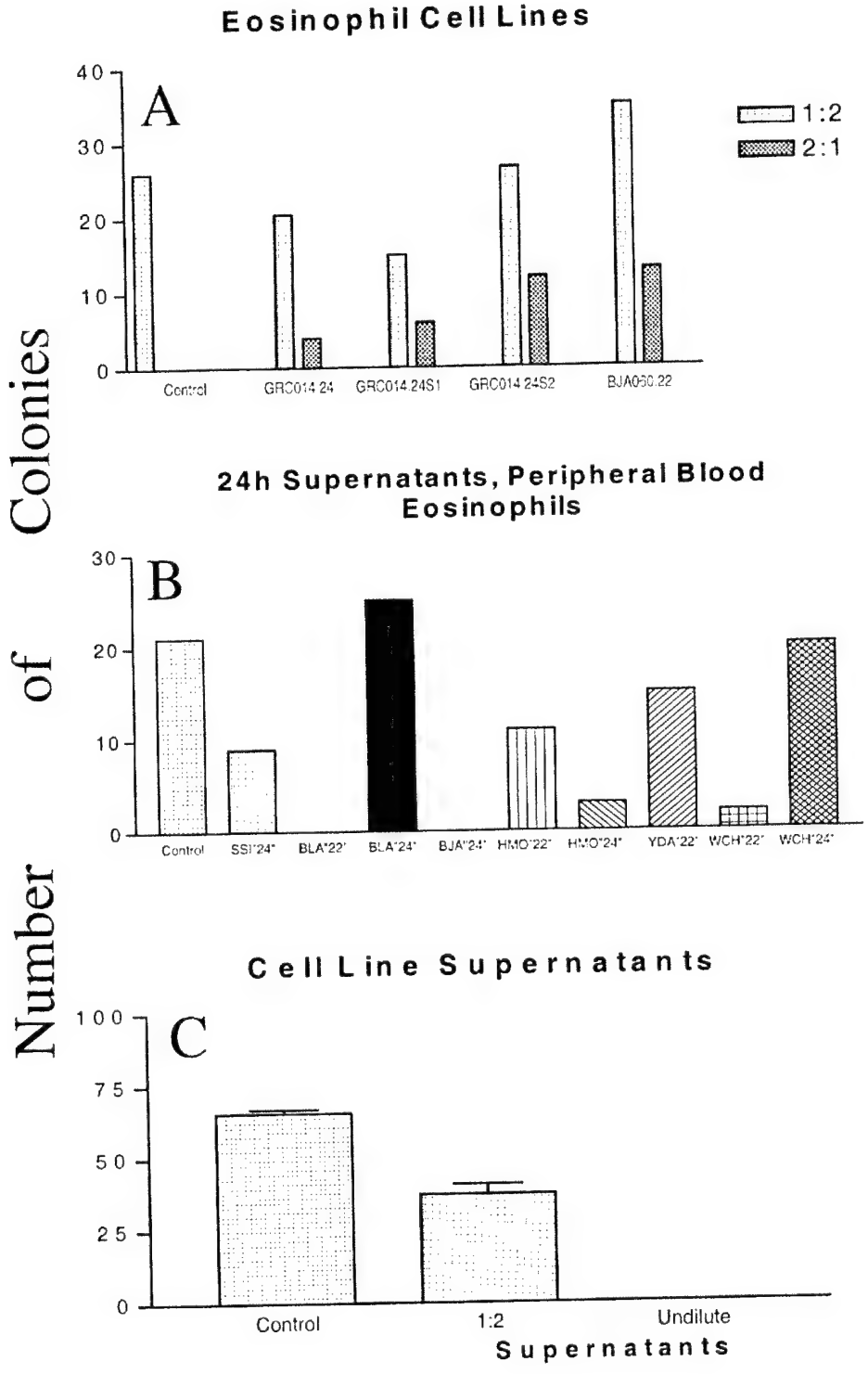


Fig. 10. DU145 cells were seeded into 6-well plates at 100 cells/well and incubated overnight at 37°C. At 24hrs. eosinophil cell lines were added at E:T ratios of 1:2 and 2:1. The plates were then cultured for 10 days at 37°C, afterwhich the plates were stained with H & E and the colonies counted (Panel A). Parent Tumor cells were also incubated with supernatants (Panel B) from cultured eosinophils from various donors and also from a cultured eosinophil cell line (Panel C).

Fig. 11. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines

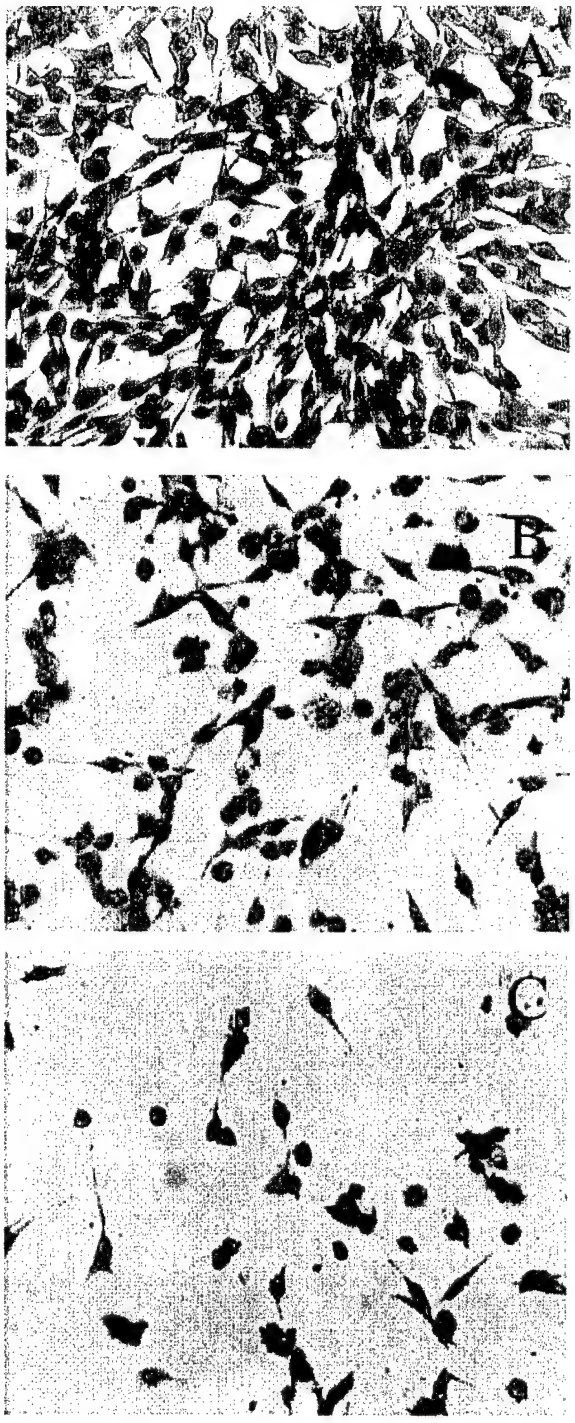
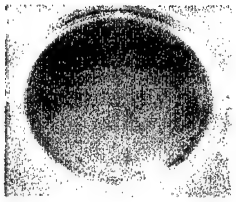
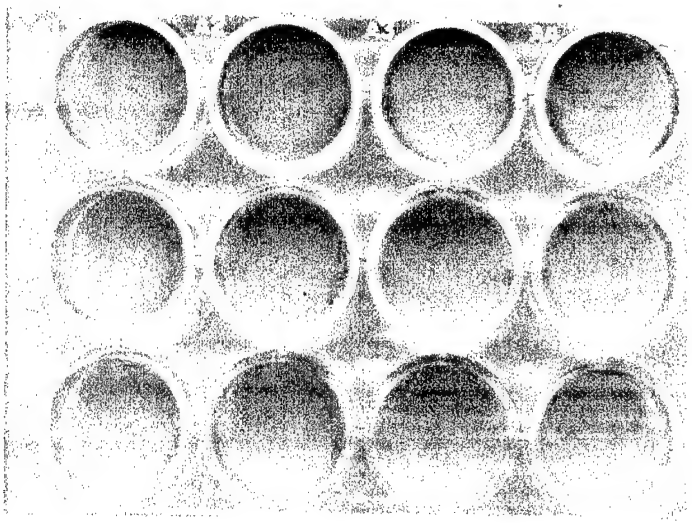


Fig. 11. HPC1 cells were seeded into the wells of a 12-well tissue cluster plate at $1.5x10^5$ cells/well. Eosinophil cell lines (IL-5 treated and untreated) were added 24hrs. later the E:T ratio of 10:1. The plates were further incubated for 24-48hr., then harvested and photomicrographs taken.

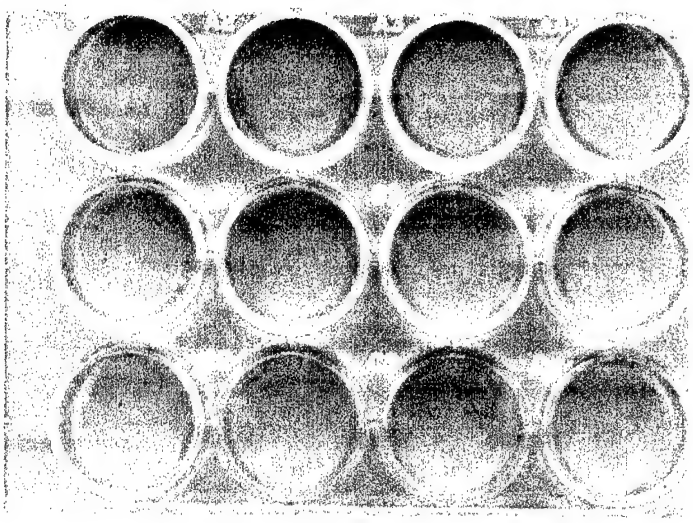
Fig. 12. HPCI Prostate Cell Growth Inhibition by Eosinophil Hypodense and Hyperdense Cell Lines



Control



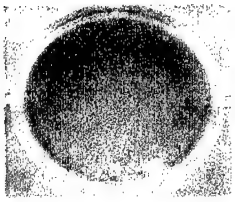
A1-2: GRC014"24":HPCI, 1:1
B1-2: GRC014"24":HPCI, 10:1
C1-2: GRC014"24":HPCI, 25:1
A3-4:GRC014"24"+IL-5:HPCI, 1:1
B3-4:GRC014"24"+IL-5:HPCI, 10:1
C3-4:GRC014"24":+IL-5HPCI, 25:1



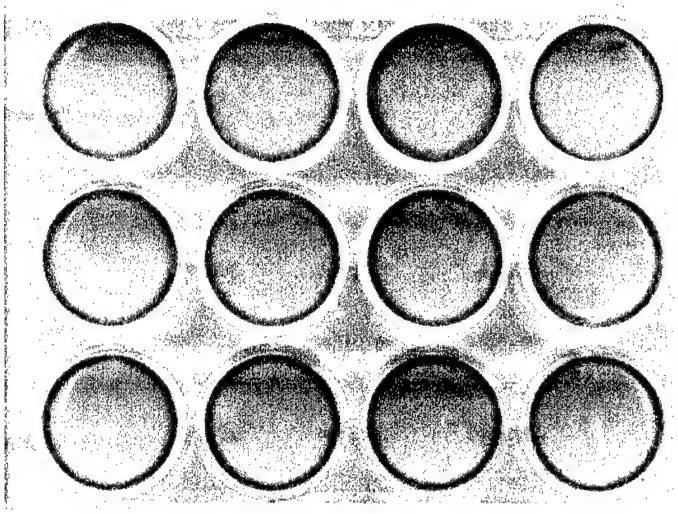
A1-2: BJA060"22":HPCI, 1:1
B1-2: BJA060"22":HPCI, 10:1
C1-2: BJA060"22":HPCI, 25:1
A3-4:BJA060"22"+IL-5:HPCI, 1:1
B3-4:BJA060"22"+IL-5:HPCI, 10:1
C3-4:BJA060"22"+IL-5:HPCI, 25:1

Fig. 12. HPCl cells were seeded into duplicate wells of a 12-well tissue culture plate similarly to that described in fig. 11. Effector cells (IL-5 treated and untreated) were added at E:T ratios of 1:1, 10:1 and 25:1. The plates were stained and scanned into power point.

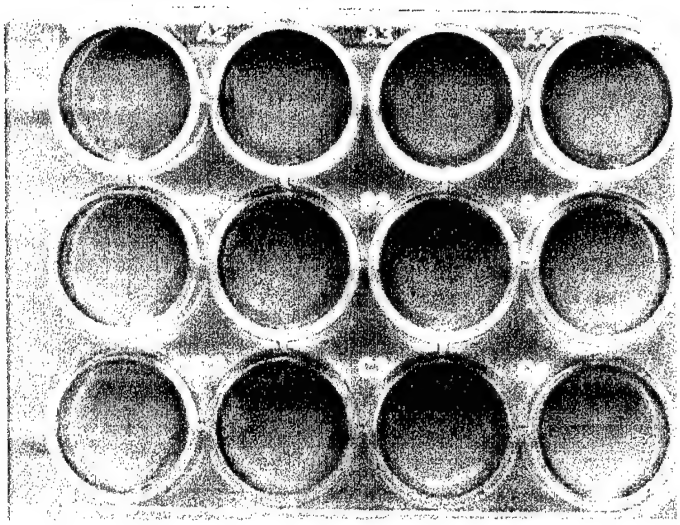
Fig 13. 24hr Eosinophil Cultured Supernatants Inhibit HPCI Cell Growth In Vitro



Control

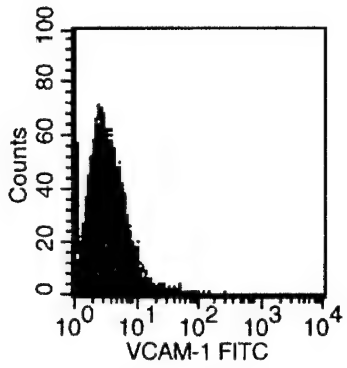


A1-2: BLA"22"
A3: BLA"24"
A4: HMO"24"
B1-2: HMO"22"
B3-4: YDA"22"
C1-2: YDA"24"
C3-4: WCH"22"

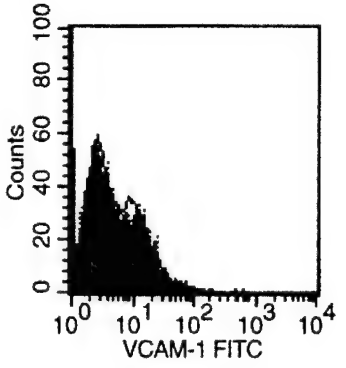


A1-2: +IL-4 @ 10ng/ml
A3-4: +IL-4 @ 50ng/ml
B1-2: +IL-4 @ 100ng/ml
B3-4: +TNF-alpha @ 10ng/ml
C1-2: +TNF-alpha @ 50ng/ml
C3-4: +TNF-alpha @ 100ng/ml

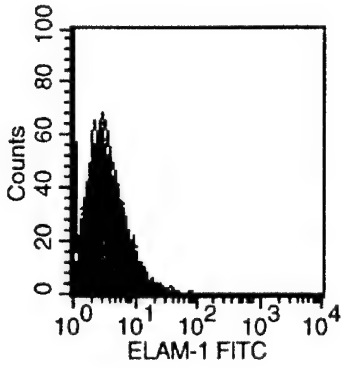
Fig. 13. HPC1 cells ($1.5x10^5$/well) were cultured for 24hrs. were incubated for an additional 24-48hrs with cultured eosinophil supernatants (Panel A) and with IL-4 and TNFα (Panel B). The plates were harvested, stained with H & E and scanned into power point.



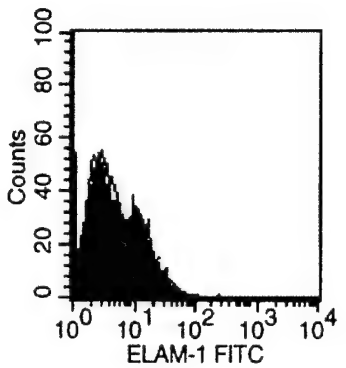
DU145 Untreated



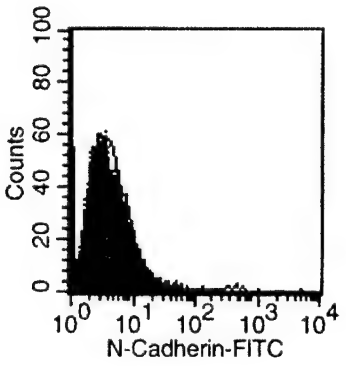
DU145 + Eos.



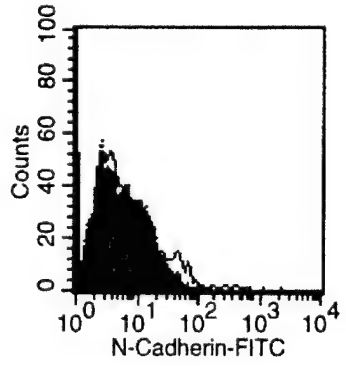
DU145 Untreated



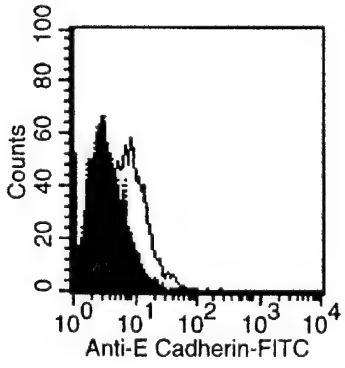
DU145 + Eos.



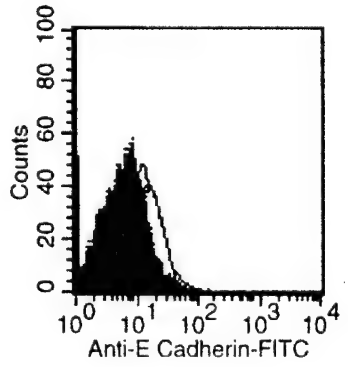
DU145 Untreated



DU145 + Eos.



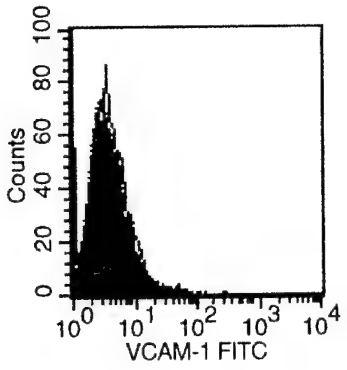
DU145 Untreated



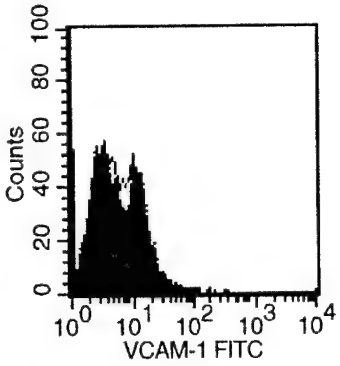
DU145 + Eos.

Figure 14: Comparison between untreated DU145 and treated DU145 with eosinophils for 24hrs at 1:1 ratio. The bottom row shows up-regulation of E Cadherin expression in the presence of eosinophils.

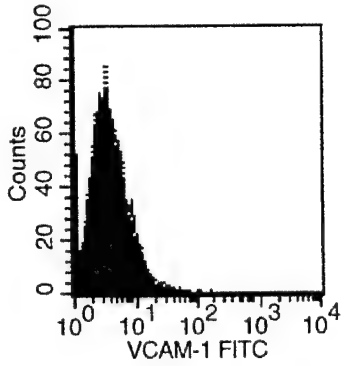
Test's control ■ Test □



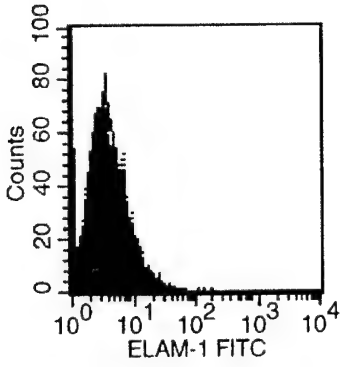
PC-3 Untreated



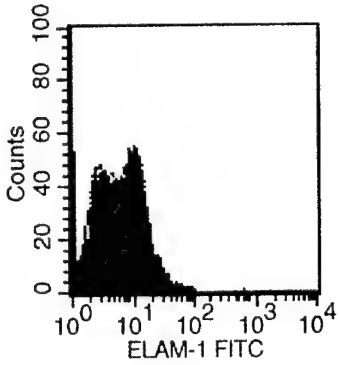
PC-3 + Eos.



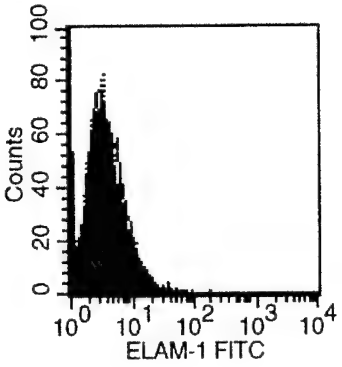
PC-3 + 24hrs Sup.



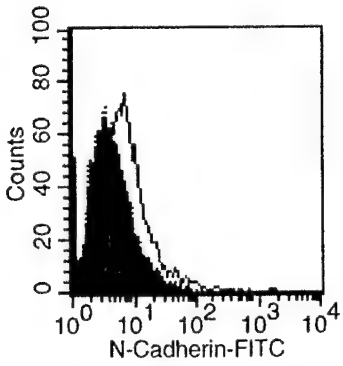
PC-3 Untreated



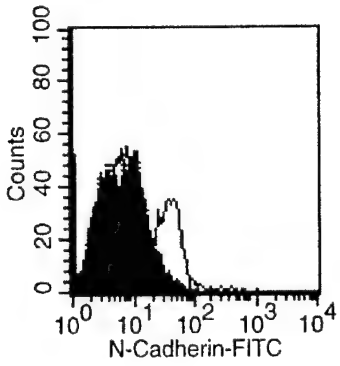
PC-3 + Eos.



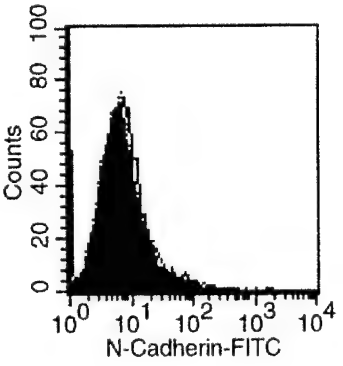
PC-3 + 24hrs Sup.



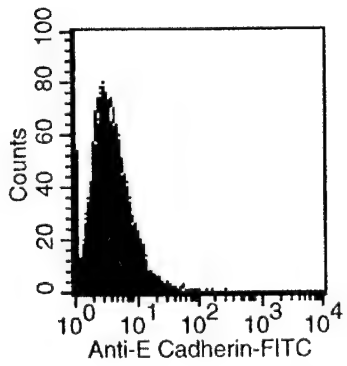
PC-3 Untreated



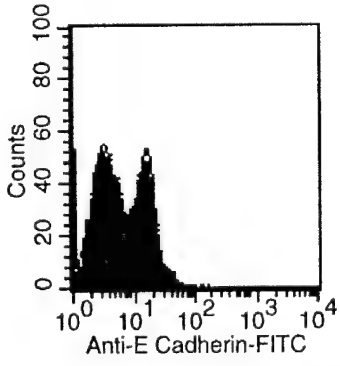
PC-3 + Eos.



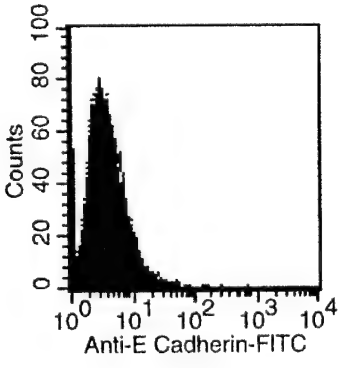
PC-3 + 24hrs Sup.



PC-3 Untreated



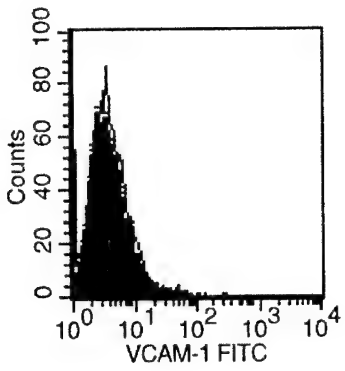
PC-3 + Eos.



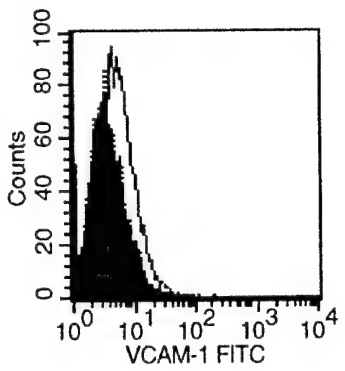
PC-3 + 24hrs Sup.

Figure15: Comparison among PC-3 untreated, PC-3 treated 24hrs with eosinophils(1:1 ratio) and PC-3 treated 24hrs with 24hrs eosinophils' supernatant. No significant change was detected.

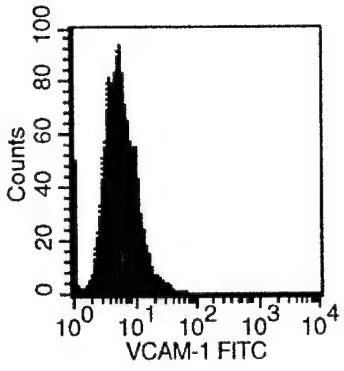
Test's control ■ Test □



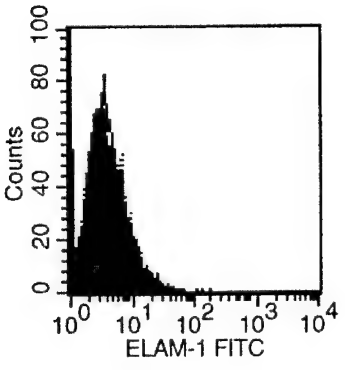
PC-3 Untreated



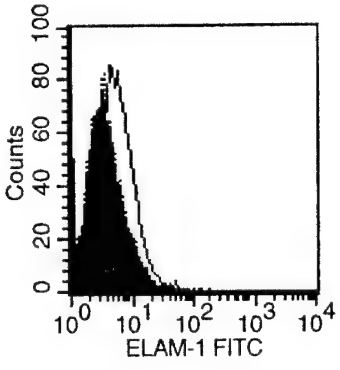
PC-3 + IL-10



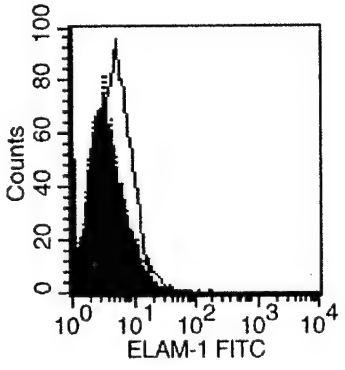
PC-3 + IL-12



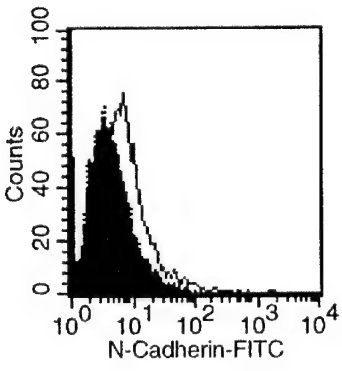
PC-3 Untreated



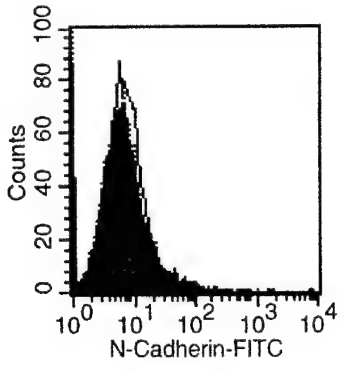
PC-3 + IL-10



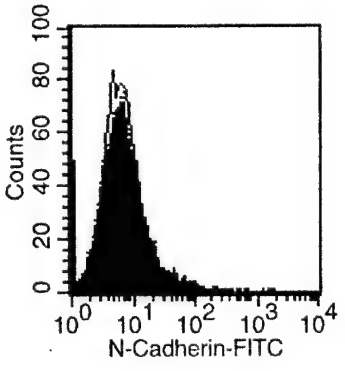
PC-3 + IL-12



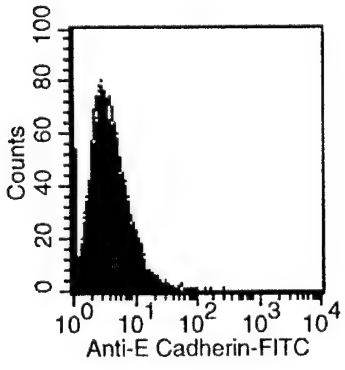
PC-3 Untreated



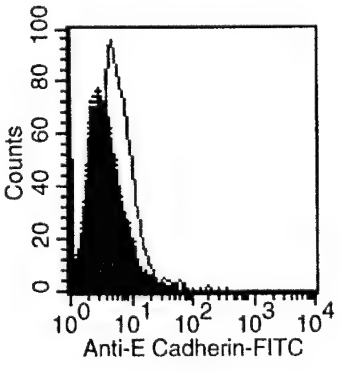
PC-3 + IL-10



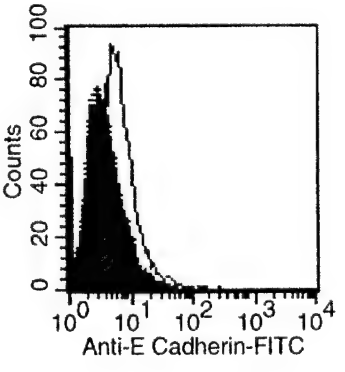
PC-3 + IL-12



PC-3 Untreated



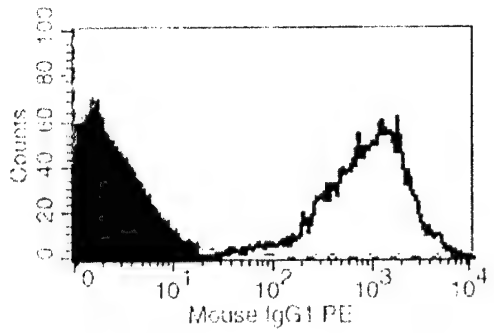
PC-3 + IL-10



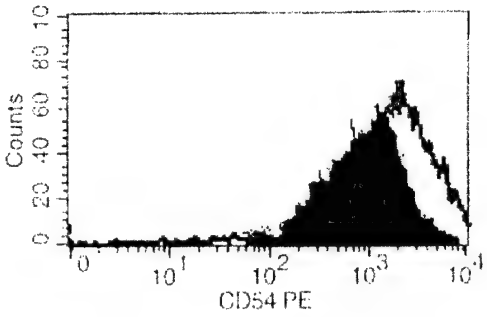
PC-3 + IL-12

Figure16: Comparison among PC-3 untreated, PC-3 treated 24hrs with 10ng/ml of IL-10 and PC-3 treated 24hrs with 10ng/ml of IL-12. IL-10 up regulated VCAM, ELAM and E Cadherin whereas IL-12 up-regulated ELAM and E Cadherin only.
Test's control ■ Test □

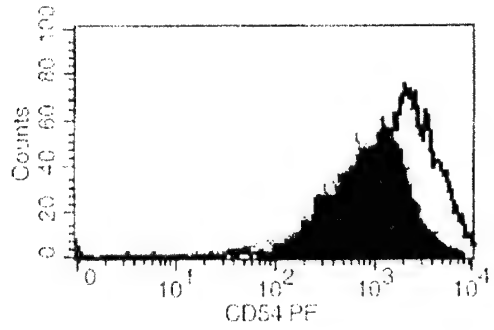
DU145



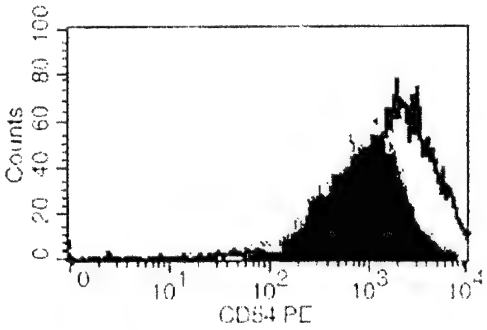
Isotype Control (■) vs. ICAM-1



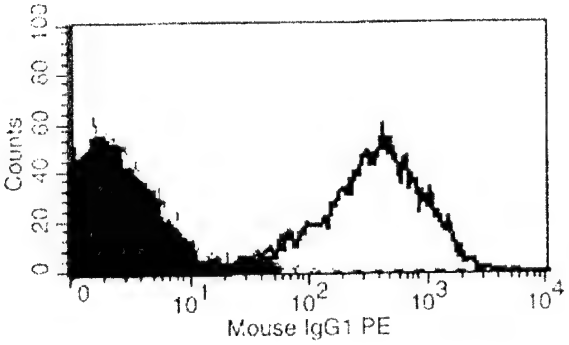
Untreated (■) vs. +TNF-a @ 1ng/ml



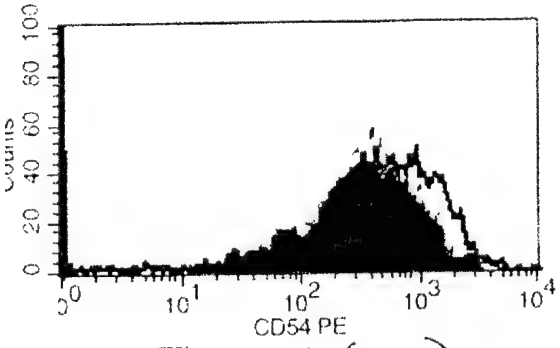
Untreated (■) vs. +TNF-a @ 10ng/ml



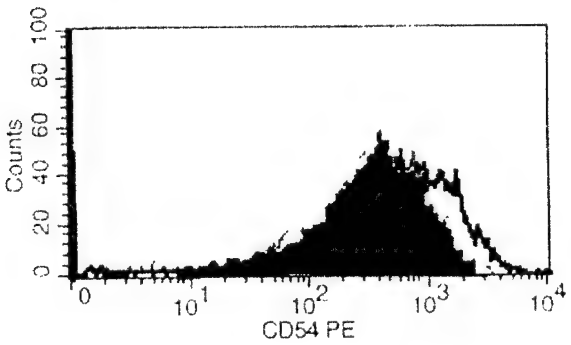
Untreated (■) vs. +TNF-a @ 100ng/ml



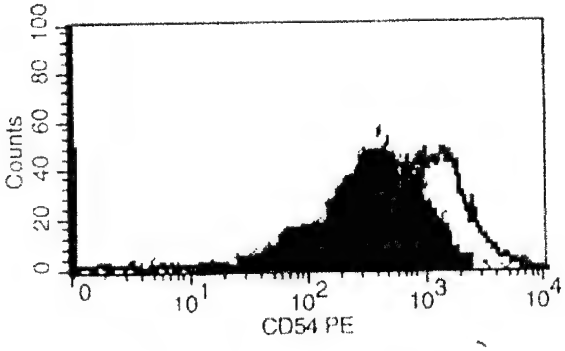
Isotype Control (■) vs. ICAM-1



Untreated (■) vs. +IL-10 @ 1ng/ml



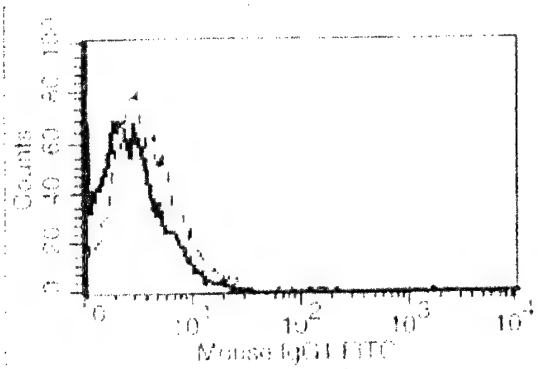
Untreated (■) vs. +IL-10 @ 10ng/ml



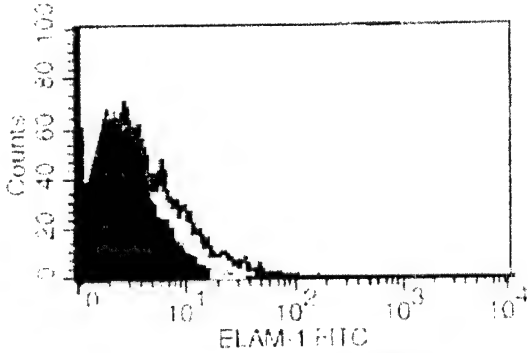
Untreated (■) vs. +IL-10 @ 100ng/ml

Figure 17: Effect of TNF-α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on DU145 prostate cancer cell line expression of ICAM-1.

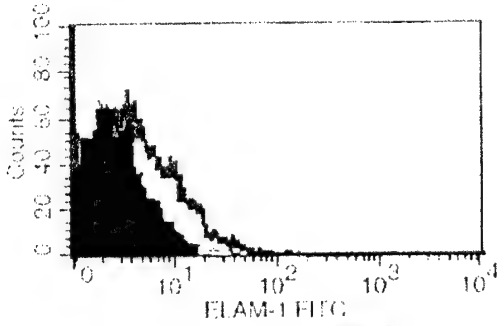
DU145



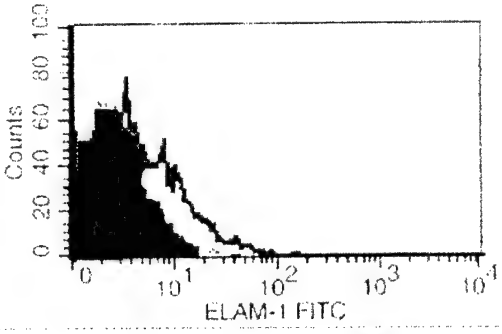
Isotype Control (--) vs. ELAM-1



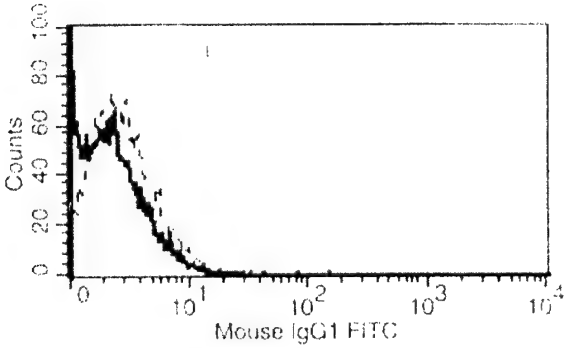
Untreated (■) vs. +TNF-a @ 1ng/ml



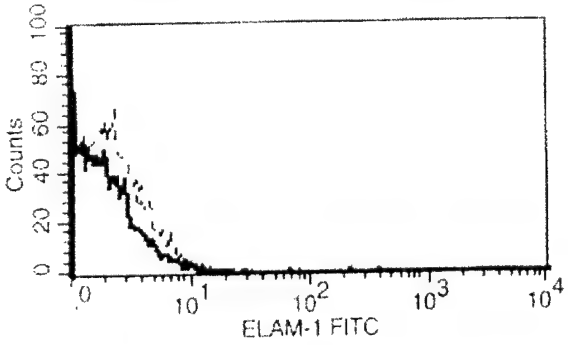
Untreated (■) vs. +TNF-a @ 10ng/ml



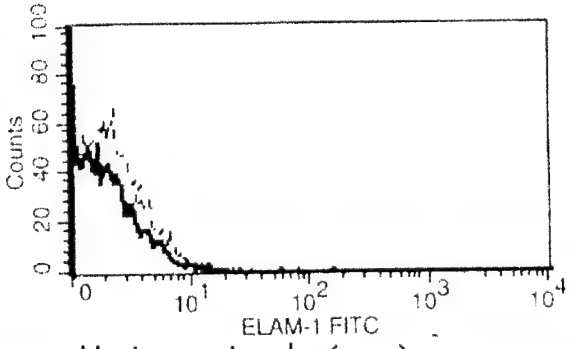
Untreated (■) vs. +TNF-a @ 100ng/ml



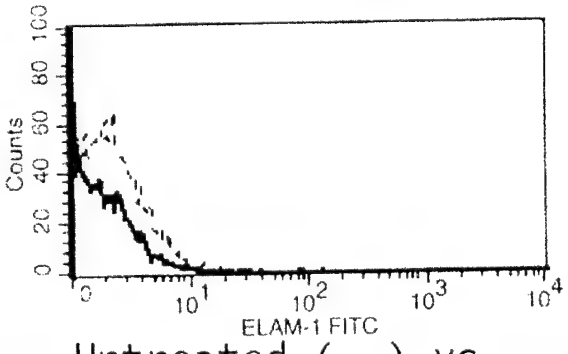
Isotype Control (--) vs. ELAM-1



Untreated (--) vs. +IL-10 @ 1ng/ml



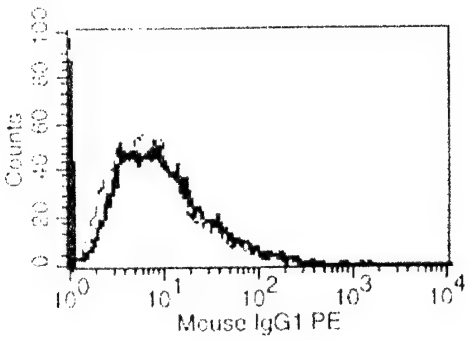
Untreated (--) vs. +IL-10 @ 10ng/ml



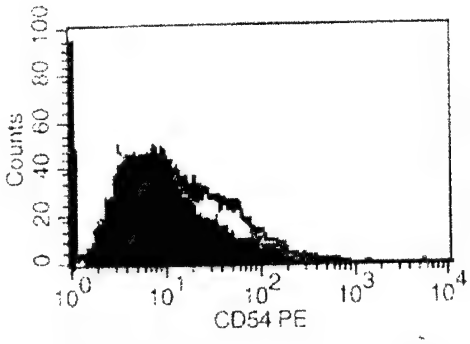
Untreated (--) vs. +IL-10 @ 100ng/ml

Figure 18: Effect of TNF-α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on DU145 prostate cancer cell line expression of ELAM-1.

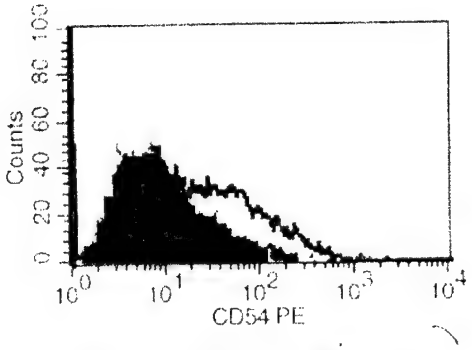
LNCaP



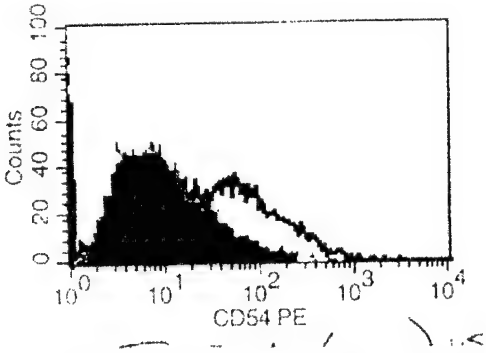
Isotype Control (--) vs.
ICAM-1



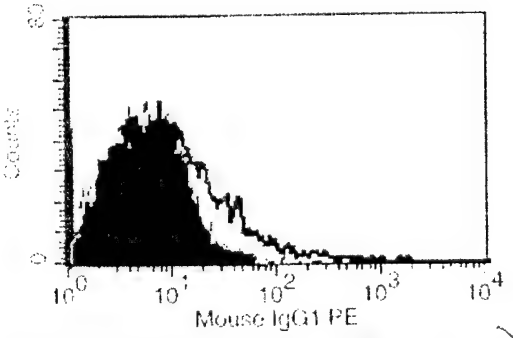
Untreated (■) vs.
+TNF-a @ 1ng/ml



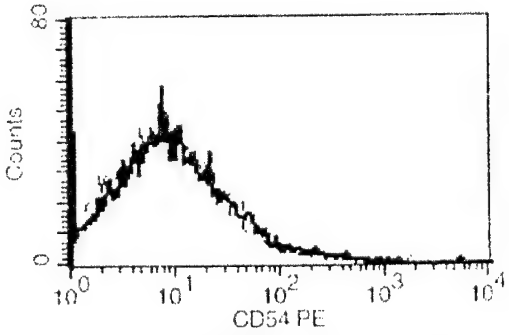
Untreated (■) vs.
+TNF-a @ 10ng/ml



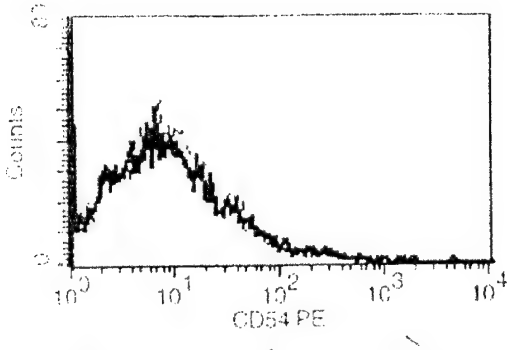
Untreated (■) vs.
+TNF-a @ 100ng/ml



Isotype Control (■) vs.
ICAM-1



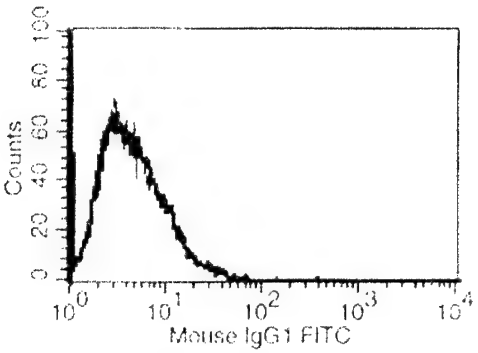
Untreated (--) vs.
+IL-10 @ 1ng/ml



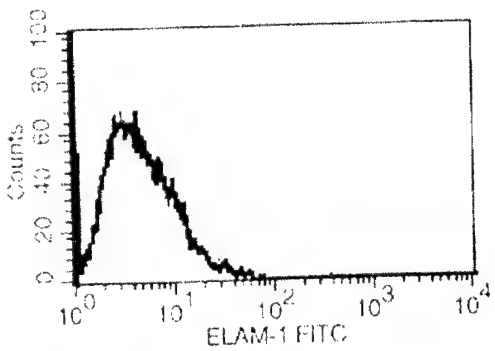
Untreated (--) vs.

Figure 19: Effect of TNF-α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on LNCaP prostate cancer cell line expression of ICAM-1.

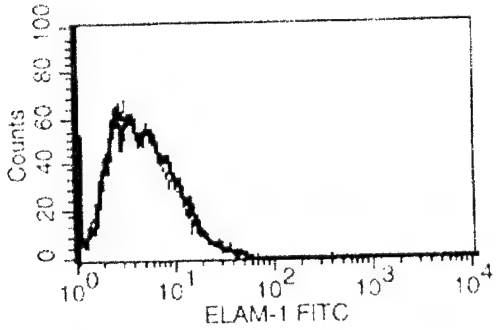
- LNCaP



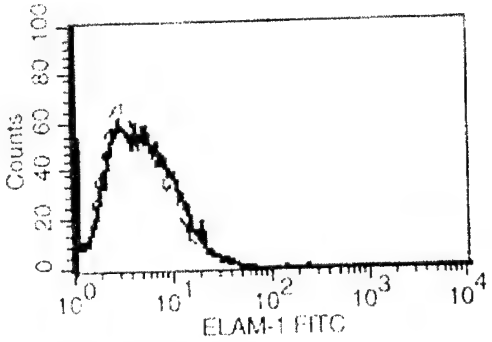
Isotype Control (--) vs. ELAM-1



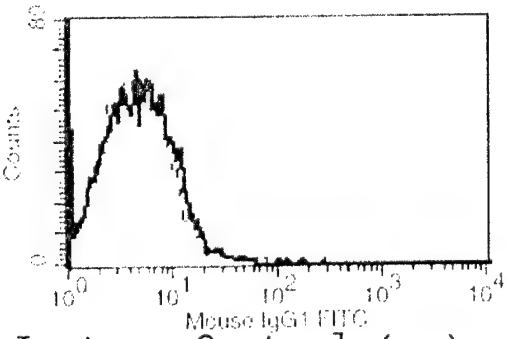
Untreated (--) vs. +TNF-a @ 1ng/ml



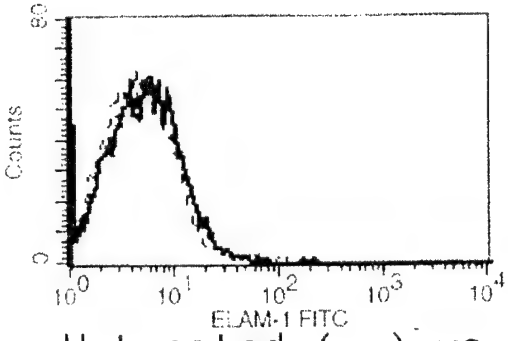
Untreated (--) vs. +TNF-a @ 10ng/ml



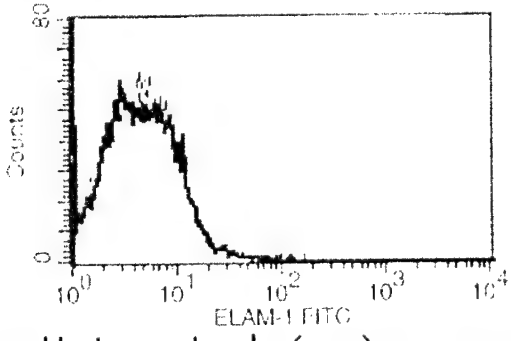
Untreated (--) vs. +TNF-a @ 100ng/ml



Isotype Control (--) vs. ELAM-1



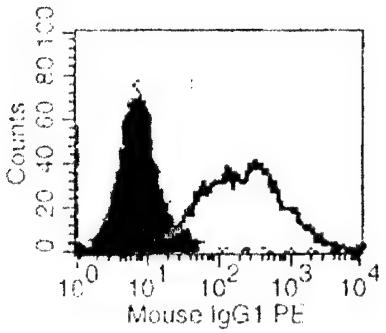
Untreated (--) vs. +IL-10 @ 1ng/ml



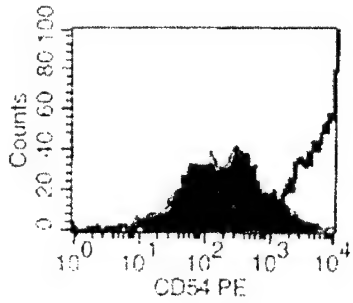
Untreated (--) vs. + IL-10 @ 10ng/ml

Figure 20: Effect of TNF-α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on LNCaP prostate cancer cell line expression of ELAM-1.

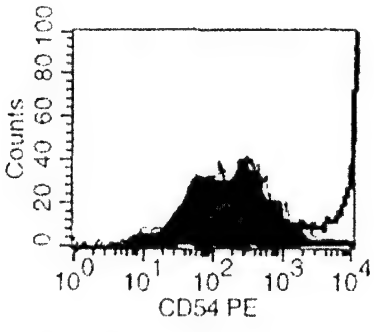
. . PC-3



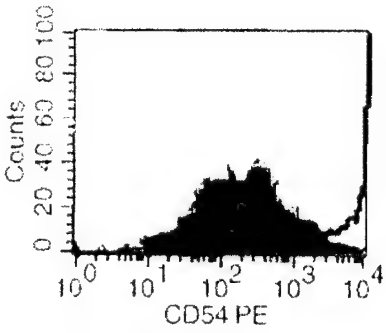
Isotype Control (■) vs.
ICAM-1



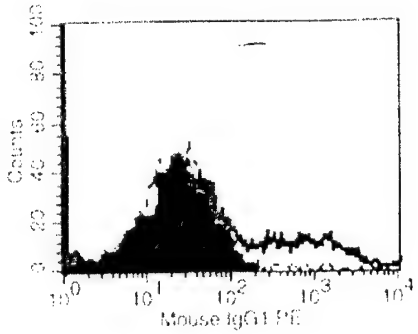
Untreated (■) vs.
+TNF-a @ 1ng/ml



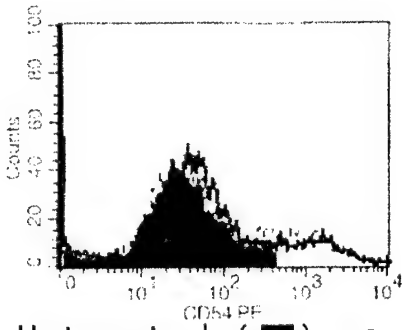
Untreated (■) vs.
+TNF-a @ 10ng/ml



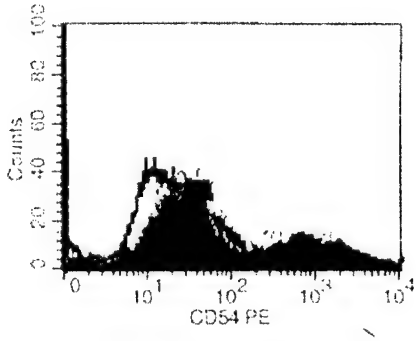
Untreated (■) vs.
+TNF-a @ 100ng/ml



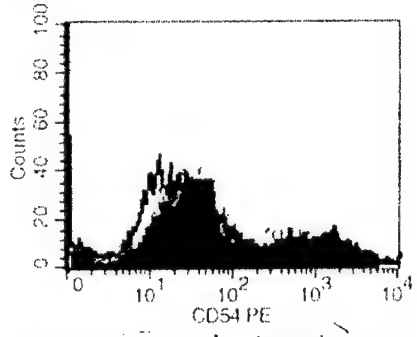
Isotype Control (■) vs.
ICAM-1



Untreated (■) vs.
+IL-10 @ 1ng/ml



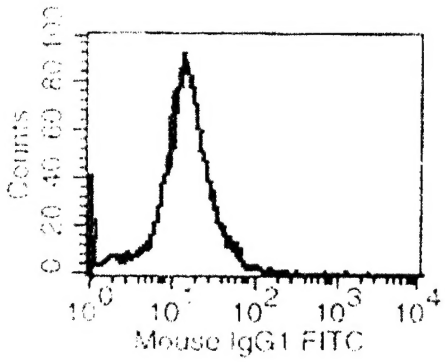
Untreated (■) vs.
+IL-10 @ 10ng/ml



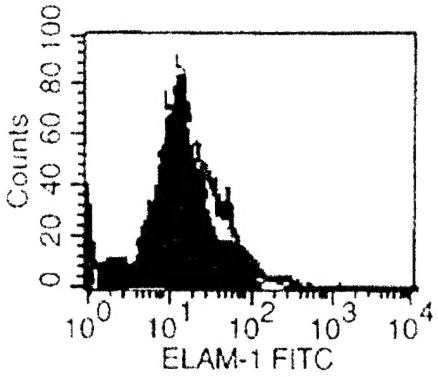
Untreated (■) vs.
+IL-10 @ 100ng/ml

Figure 21: Effect of TNF-α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on PC-3 prostate cancer cell line expression of ICAM-1.

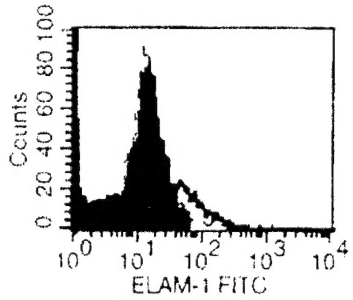
PC-3



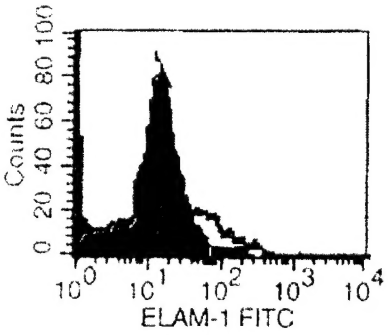
Isotype Control (--) vs.
ELAM-1



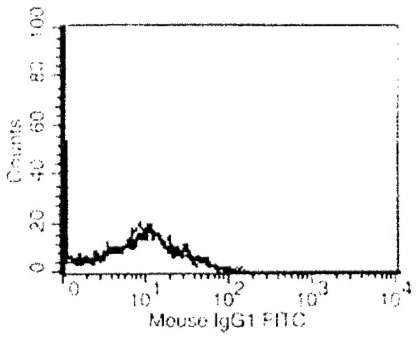
Untreated (■) vs.
+TNF-a @ 1ng/ml



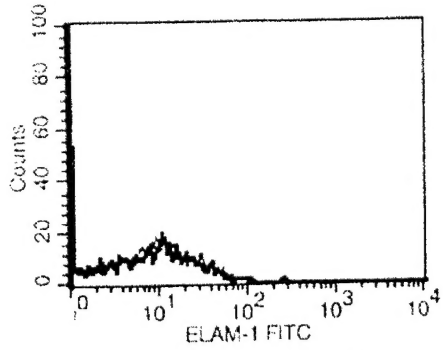
Untreated (■) vs.
+TNF-a @ 10ng/ml



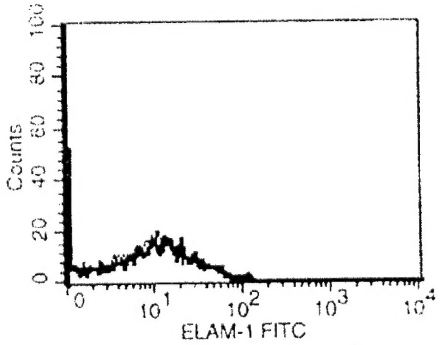
Untreated (■) vs.
+TNF-a @ 100ng/ml



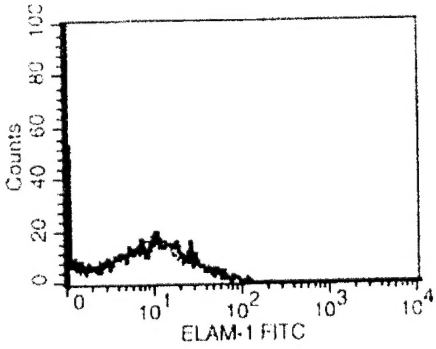
Isotype Control (--) vs.
ELAM-1



Untreated (--) vs.
+IL-10 @ 1ng/ml



Untreated (--) vs.
+IL-10 @ 10ng/ml

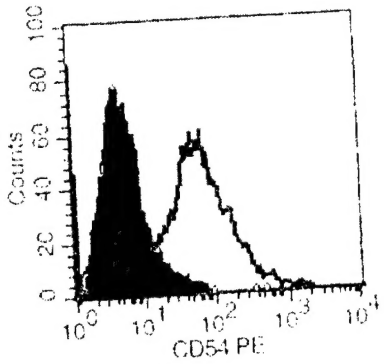


Untreated (--) vs.
+IL-10 @ 100ng/ml

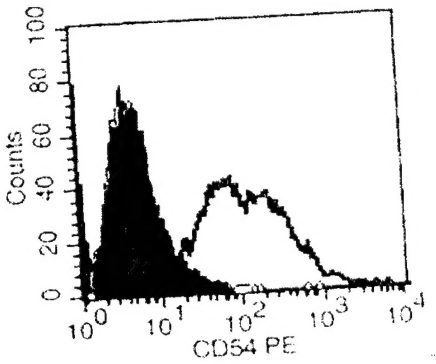
Figure 22: Effect of TNF-α and IL-10 at 1ng/ml, 10ng/ml, and 100ng/ml on PC-3 prostate cancer cell line expression of ELAM-1.

PC-3

Fig. 23

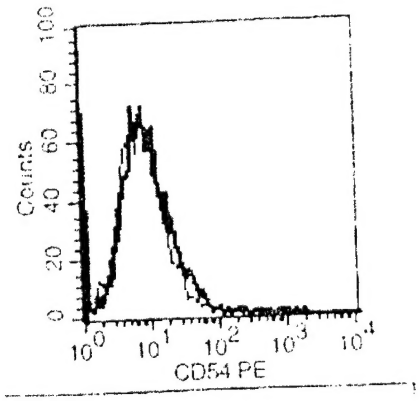


Isotype Control (■) vs.
ICAM-1

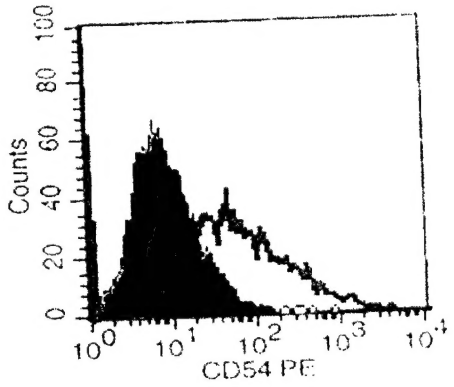


Isotype Control (■) vs.
+IL-1

LNCaP

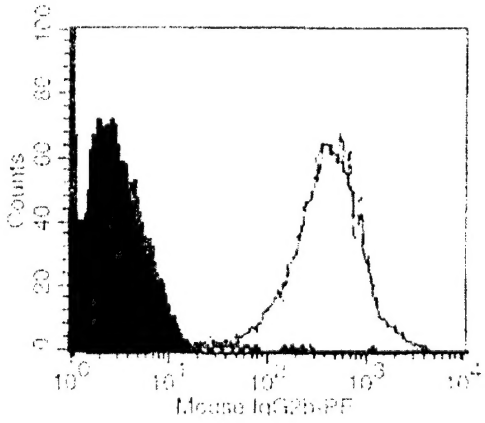


Isotype Control (--) vs.
ICAM-1

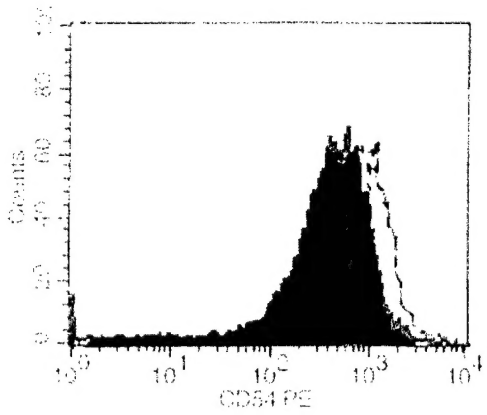


Isotype Control (■) vs.
+IL-1

DU145



Isotype Control (■) vs.
ICAM-1



Untreated (■) vs.
+IL-1

Figure 23: Effect of IL-1α on PC-3, LNCaP, and DU145 prostate cancer cell lines expression of ICAM-1.